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Project Report: Bacterial Adaptation to Low Temperatures

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Project Progress

This project uses experimental evolution to adapt lineages of the bacterium *Escherichia coli* to low temperature, and examines the genetic basis and the functional consequences of that adaptation. We have previously developed a primary set of six clonal lineages and a secondary set of an additional 24 lineages that have adapted to 20°C. We are in the process of developing six new lineages adapted to 14°C.

We are currently conducting a proteomic study that examines differences between the primary six lines adapted to 20°C and their common ancestor. The analysis is preliminary, but intriguing differences have emerged between the cold adapted lineages and warm adapted ancestor. Specifically, levels of two ribosomal proteins as well as two elongation factors have been altered, suggesting that the translational machinery may be a target of selection during cold adaptation. Additionally, changes have been found in a PTS component potentially involved with glucose transport, and in several proteins that appear to be temperature related, including two chaperone proteins (HSP70 and TF), a fatty-acid related enzyme, and a DNA-binding protein reported to have thermal specificity.

In a continuing analyses of evolutionary tradeoffs, we examined performance at high temperature (40 – 41 °C) in the secondary group of 24 lineages adapted to low temperature. Competitive fitness at 20°C improved significantly, whereas competitive fitness and cell yield at 40°C declined significantly for the evolved group as a whole. However, there was considerable variation among the low-temperature evolved lines in their performance at high temperature, suggesting that significant tradeoffs need not necessarily occur as part of the low temperature adaptive process.

Insights have also been gained into evolutionary adaptation to freezing and thawing conditions in *E. coli*. We previously demonstrated substantial mortality during repeated freeze-thaw cycles in the absence of cryoprotectant, and populations evolved at 37°C were more sensitive than were their ancestors. Based on these findings, we designed a 1,000-generation experiment to

investigate the potential for evolutionary adaptation to alternating periods of freezing, thawing, and growth at 37°C. The evolution phase of this experiment was completed this past year, and work is now underway to analyze phenotypic and genetic changes in the freeze/thaw/growth evolved lines relative to their ancestors. Competition assays between the evolved lines and their ancestors indicate that significant genetic adaptation has indeed occurred. Improved survival during the freeze/thaw phase, and a shorter lag prior to the start of exponential growth, are two major demographic changes underlying this adaptation.

Highlights

- Proteomic analyses indicate that protein translation may be an important target of selection during cold adaptation.
- During low temperature adaptation, significant loss of performance at high temperature usually, but not universally, occurs.
- Completed an experiment in which *E. coli* populations evolved for 1,000 generations in an environment subject to alternating periods of freezing, thawing, and growth. Competitions between the evolved lines and their ancestors confirm significant adaptation, with improved survival during the freeze/thaw phase as well as a faster transition to growth after thawing.

Roadmap Objectives

- **Objective No. 4.2:** Foundations of complex life
- **Objective No. 5.1:** Environment-dependent, molecular evolution in microorganisms
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth